Pestic. Sci. 1997, **50**, 240–248

Status of Insecticide Resistance in *Spodoptera litura* in Andhra Pradesh, India

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(Received 28 October 1996; accepted 3 March 1997)

Abstract: Twenty-two strains of the tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), collected from groundnut crops of eight locations in Andhra Pradesh, India, between 1991 and 1996 were assayed in the F1 generation for resistance to commonly used insecticides. Resistance levels ranged as follows: cypermethrin, 0·2- to 197-fold; fenvalerate, 8- to 121-fold; endosulfan, 1- to 13-fold; quinalphos, 1- to 29-fold; monocrotophos, 2- to 362-fold and methomyl, 0·7- to 19-fold. In nearly all strains pre-treatment with the metabolic inhibitor, piperonyl butoxide, resulted in complete suppression of cypermethrin resistance (2- to 121-fold synergism), indicating that enhanced detoxification by microsomal P450-dependent monooxygenases was probably the major mechanism of pyrethroid resistance. Pre-treatment with the synergist DEF, an inhibitor of esterases and the glutathione *S*-transferase system, resulted in a 2- to 3-fold synergism with monocrotophos indicating that esterases and possibly glutathione *S*-transferases were at least to some extent contributing to organophosphate resistance.

Pestic Sci., **50**, 240–248, 1997
No. of Figures: 1. No. of Tables: 9. No. of Refs. 15

Key words: *Spodoptera litura*, pyrethroids, organophosphates, carbamates, endosulfan, resistance

1 INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (F.) is a polyphagous noctuid of high reproductive capacity with an ability to migrate over large distances in the adult stage. As with other members of the genus, these characteristics have resulted in it becoming a pest of many agricultural crops throughout its geographical range, which extends throughout Asia and Oceania, from the borders of North Africa to Japan and New Zealand. In India it has become particularly notorious for the damage it causes to tobacco in most of the tobacco-growing tracts of the country.[1] However, during the last 30 years it has become increasingly important on other crops—cotton, groundnut and mung bean in particular.

S. litura was one of the first pests of agricultural importance in India to develop resistance to insecticides. By 1965 resistance to benzene hexachloride (BHC) was reported in field populations from Rajasthan[2] and by the early 1970s to endosulfan and carbaryl in Haryana[3] and West Bengal.[4] In the early 1980s, populations in the south Indian state of Andhra Pradesh were shown to be highly resistant to lindane, endosulfan, carbaryl and malathion.[5]

It was largely as a result of serious control difficulties caused by insecticide-resistant *S. litura* that the synthetic pyrethroids were approved for application to

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Contract grant sponsor: Natural Resources Institute, UK.

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

cotton and released into the Indian market-place in 1982. The pyrethroids were effective to the extent that, by the 1983–84 season, this species had been relegated to the status of a minor cotton pest. Unfortunately, since this time, *S. litura* has become an increasingly important pest of groundnut, particularly in the east coastal region of peninsular India which accounts for 15% of the country's annual production of 8·5 Mt (million tonnes). By the late 1980s it was widely considered to be out of control by groundnut farmers in the region and the research-extension sector alike.[6]

This study was undertaken to determine whether the status of insecticide resistance in *S. litura* in Andhra Pradesh had changed since the study by Ramakrishnan *et al.*[5] and whether resistance to the synthetic pyrethroids and some of the more recently introduced organophosphate and carbamate insecticides had developed. These data would aid our understanding of recent control failures with insecticides in groundnut crops, the reason for the increasing status of *S. litura* as a pest of groundnut in the coastal Andhra Pradesh districts and serve as a bench-mark against which to measure the success of IPM programmes being implemented in the region.

2 EXPERIMENTAL METHODS

2.1 Sample collection and rearing

S. litura egg masses were collected from groundnut crops in farmers' fields at eight locations in Andhra Pradesh State and from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) farm, Hyderabad, between 1991 and 1996 (Fig. 1 and Table 1). All strains, with the exception of one from the ICRISAT farm (from groundnut in July 1993 during the rainy season), were collected from post-rainy season groundnut crops during January and February. Wherever possible, farmers were interviewed to determine the number of insecticide sprays applied to their crop up to the time of sampling. In the laboratory, larvae were reared on a semi-synthetic diet based on chickpea flour and dried ground sorghum leaves (Ranga

Fig. 1. Sampling locations for *Spodoptera litura* strains in Andhra Pradesh, India between 1991 and 1996.

TABLE 1
Sources of *Spodoptera litura* Strains from Andhra Pradesh State between 1991 and 1996 and Numbers of Applications of Insecticide to Groundnut Crops prior to Collection of Egg Masses

Location, district	*Collection date*	*No. of insecticide sprays*
ICRISAT, Medak	January 1991	0
ICRISAT, Medak	July 1993	0
ICRISAT, Medak	February 1995	0
Tiruvuru, Krishna	January 1991	1–2
Tiruvuru, Krishna	February 1993	2
Bapatla, Guntur	February 1991	6–7
Bapatla, Guntur	February 1994	2–3
Bapatla, Guntur	February 1995	0
Bapatla, Guntur	February 1995	1–2
Bapatla, Guntur	February 1996	0
Bapatla, Guntur	February 1996	10
Karlapalem, Guntur	February 1993	5–6
Gonapavarum, Guntur	February 1993	6–7
Kottapatnam, Prakasam	February 1991	1–2
Proddatur, Cuddapah	February 1995	0
Proddatur, Cuddapah	February 1995	5–9
Proddatur, Cuddapah	February 1996	0
Proddatur, Cuddapah	February 1996	2
Tirupati, Chittoor	February 1995	0
Tirupati, Chittoor	February 1995	4–5
Tirupati, Chittoor	February 1996	0
Tirupati, Chittoor	February 1996	2–3

Rao, unpublished). All rearing operations were conducted at 25(±2)°C under natural photoperiod (*c.*13 : 11 h light : dark). An insecticide-susceptible strain of *S. litura* was obtained from Rallis India Ltd. This strain was originally collected from volunteer castor plants from the Bangalore region and had been maintained in the laboratory for six years.

2.2 Insecticides

The following technical grade insecticides were used for bioassyas: *cis : trans* (*c.*50 : 50 ratio) cypermethrin (900 g kg^{-1}; Zeneca Agrochemicals, UK); endosulfan (940 g kg^{-1}; Excel Industries, India); fenvalerate (976 g kg^{-1}; Sumitomo Corp., Japan); methomyl (980 g kg^{-1}; DuPont, France); monocrotophos (680 g kg^{-1}; Khatau Junker, India); quinalphos (720 g kg^{-1}; Sandoz, India). The synergists, piperonyl butoxide (PBO) (900 g kg^{-1}) and *S,S,S*-tributylphosphorotrithionate (DEF), were obtained from Goodeed Chemical Co. Ltd, UK, and Mobay Chemical Co., USA, respectively.

2.3 Bioassay and data analysis

Biossays were conducted on 30–40-mg F1 generation larvae using a procedure based on the standard *Heliothis* susceptibility test recommended by the Entomological Society of America.[7] Serial dilutions of technical grade insecticides in analytical grade acetone were prepared and a 1·0-μl drop dispensed onto the thoracic dorsum of individual larvae using a Hamilton® repeating dispenser fitted with a 50-μl syringe. Control larvae were treated with acetone alone. For each bioassay generally at least 36 larvae were treated at each of five or more concentrations, plus control. In assays including the synergists PBO and DEF, these were applied as 1·0-μl drops to the mesothorax 15–20 min prior to the insecticide, at rates of 50 μg per larva and 20 μg per larva respectively. After dosing, larvae were held individually in 7·5-ml cells of 12-well tissue culture plates (Linbro, ICN Flow Ltd) with fresh semi-synthetic diet.

Mortality was assessed six days (144 h), after treatment. A larva was considered 'dead' if it was unable to move in a co-ordinated manner when prodded with a blunt needle. Control mortality was rare, but where necessary corrections were made using Abbott's formula.[8]

Dose mortality regressions were computed by probit analysis using MLP 3.08 software.[9] Significance of differences between log dose probit (ldp) lines was determined from Position χ^2 test (to determine whether relative potencies differ from unity), and Parallelism χ^2 test (to determine whether a common slope is adequate). Heterogeneity χ^2 tests were performed on all ldp lines to determine whether or not residual variation was consistent with binomial sampling.[9]

3 RESULTS

3.1 Insecticide use

Insecticide use was highly variable both within sampling regions and from one year to the next (Table 1). There were always some farmers who did not use insecticide at all for *S. litura* control, opting to follow ICRISAT and State extension advice, particularly since the 1993–94 season. However, the majority of farmers still continued to use insecticide and the number of applications ranges from 1 to 10, depending upon pest attack and farmers' perceptions of insect pest damage. Typical insecticides used on post-rainy season groundnut were: lindane, chlorpyrifos, cypermethrin, endosulfan, fenvalerate, parathion-methyl, monocrotophos and quinalphos. Dust formulations of lindane, fenvalerate, parathion-methyl (folidol) and quinalphos were popular because of their long residual action against leaf feeding pests such as *S. litura.*

3.2 Pyrethroid resistance

The steep ldp line slopes (>2·1), relatively low LD_{50} values and, in the case of cypermethrin, no suppression

TABLE 2
Toxicity of Topically Applied Cypermethrin to 30–40-mg Larvae of Field Strains of *Spodoptera litura*

Strain[a]	*Collect date*	n	LD_{50}[c] (*95% C.I.*) (*μg per larva*)		LD_{90}	*Slope* (±*S.E.*)	*RF*[b]
Bangalore		288	0·029	(0·02–0·03)	0·091	2·6 (±0·3)	—
ICRISAT (US)	Jan. 91	198	0·022*	(0·01–0·04)	0·26	1·2 (±0·2)	0·8
ICRISAT (US)	Jul. 93	257	0·058	(0·04–0·08)	0·34	1·7 (±0·2)	2
ICRISAT (US)	Feb. 95	384	0·047	(0·03–0·06)	0·76	1·1 (±0·1)	2
Kottapatnam (S)	Feb. 91	248	0·090	(0·05–0·16)	3·1	0·83 (±0·1)	3
Karlapalem (S)	Feb. 93	480	0·0046	(0·003–0·007)	0·34	0·69 (±0·1)	0·2
Gonapavarum (S)	Feb. 93	528	0·30	(0·23–0·39)	3·8	1·2 (±0·1)	10
Tiruvuru (S)	Jan. 91	288	0·010	(0·008–0·01)	0·054	1·8 (±0·2)	0·3
Tiruvuru (S)	Feb. 93	183	0·049	(0·03–0·10)	0·29	1·7 (±0·4)	2
Bapatla (S)	Feb. 91	128	0·011	(0·006–0·02)	0·10	1·4 (±0·2)	0·4
Bapatla (S)	Feb. 94	432	0·16	(0·13–0·21)	1·0	1·6 (±0·1)	6
Bapatla (US)	Feb. 95	240	0·44*	(0·37–0·53)	1·1	3·2 (±0·5)	15
Bapatla (S)	Feb. 95	288	0·39*	(0·28–0·51)	2·5	1·6 (±0·2)	13
Bapatla (US)	Feb. 96	216	2·8	(2·2–3·6)	12	2·0 (±0·3)	97
Bapatla (S)	Feb. 96	180	5·7	(3·6–8·9)	57	1·3 (±0·2)	197
Proddatur (S)	Feb. 95	288	0·31	(0·23–0·40)	2·2	1·5 (±0·2)	11
Proddatur (US)	Feb. 96	144	3·1*	(2·6–3·8)	7·0	3·7 (±0·5)	107
Proddatur (S)	Feb. 96	180	2·8	(2·3–3·5)	8·2	2·8 (±0·4)	97
Tirupati (US)	Feb. 95	288	0·13	(0·09–0·23)	1·3	1·3 (±0.2)	4
Tirupati (S)	Feb. 95	288	0·058	(0·04–0·07)	0·33	1·7 (±0·2)	2
Tirupati (US)	Feb. 96	216	0·64	(0·51–0·79)	2·3	2·3 (±0·3)	22
Tirupati (S)	Feb. 96	180	1·5*	(1·2–2·0)	5·9	2·1 (±0·4)	52

[a] (S) = collection from sprayed fields: (US) from unsprayed fields.
[b] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain.
[c] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$.

TABLE 3
Effect of Piperonyl Butoxide in Suppressing Cypermethrin Resistance in 30–40-mg Larvae Derived from Field Strains of *Spodoptera litura*

		Cypermethrin alone		*Cypermethrin + PBO*			
Strain[a]	*Collect date*	LD_{50}[b] (*μg per larva*)	*Slope*	LD_{50}[b] (*μg per larva*)	*Slope*	*RF*[c]	*SR*[d]
Bangalore		0·029	2·6	0·039	1·7	—	0·7
ICRISAT (US)	Feb. 95	0·047	1·1	0·013	1·2	0·3	4
Bapatla (S)	Feb. 94	0·16	1·6	0·033	1·8	0·8	5
Bapatla (US)	Feb. 95	0·44	3·2	0·012	2·3	0·3	37
Bapatla (S)	Feb. 95	0·39*	1·6	0·022	1·1	0·6	18
Proddatur (S)	Feb. 95	0·31	1·5	0·011	1·2	0·3	28
Tirupati (US)	Feb. 95	0·13	1·3	0·040	0·91	1	3
Tirupati (S)	Feb. 95	0·058	1·7	0·030**	0·82	0·8	2
Bapatla (US)	Feb. 96	2·8	2·0	0·066	2·5	2	42
Bapatla (S)	Feb. 96	5·7	1·3	0·047	4·5	1	121
Proddatur (US)	Feb. 96	3·1	3·7	0·079	2·9	2	39
Proddatur (S)	Feb. 96	2·8	2·8	0·043	2·0	1	65
Tirupati (US)	Feb. 96	0·64	2·3	0·057	1·6	1	11
Tirupati (S)	Feb. 96	1·5	2·1	0·081	1·5	2	19

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.
[c] RF (resistance factor) = LD_{50} field Strain/LD_{50} Bangalore strain (both pretreated with PBO).
[d] SR (synergism ratio) = LD_{50} without PBO/LD_{50} with PBO pretreatment.

TABLE 4
Toxicity of Topically Applied Fenvalerate to 30–40-mg Larvae of Field Strains of *Spodoptera litura*

Strain[a]	*Collect date*	n	LD_{50}[b] (*95% C.I.*) (*μg per larva*)		LD_{90}	*Slope* (±*S.E.*)	*RF*[c]
Bangalore		288	0·043	(0·035–0·052)	0·17	2·2 (±0·2)	—
ICRISAT (US)	Jul. 93	336	0·82**	(0·64–1·1)	6·1	1·5 (±0·2)	19
ICRISAT (US)	Feb. 95	384	0·088**	(0·066–0·12)	1·0	1·2 (±0·1)	2
Bapatla (S)	Feb. 94	576	3·4*	(2·6–4·4)	39	1·2 (±0·1)	79
Bapatla (US)	Feb. 95	288	5·2	(3·9–6·7)	34	1·6 (±0·2)	121
Bapatla (S)	Feb. 95	288	3·0	(2·2–4·1)	29	1·3 (±0·2)	70
Proddatur (US)	Feb. 95	288	0·65***	(0·49–0·95)	5·7	1·4 (±0·2)	15
Tirupati (US)	Feb. 95	432	0·35**	(0·25–0·47)	5·2	1·1 (±0·1)	8
Tirupati (S)	Feb. 95	336	0·84**	(0·62–1·1)	8·2	1·3 (±0·2)	20

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
[c] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain.

of resistance by the metabolic inhibitor PBO (Position χ^2, $P > 0{\cdot}05$), indicate that the Bangalore strain was susceptible to cypermethrin and fenvalerate (Tables 2–4).

Cypermethrin and fenvalerate resistance in field strains ranged from 0·8- to 197-fold and 2- to 121-fold respectively. In all cases where both cypermethrin and fenvalerate were assayed on the same strains, resistance was significantly greater for fenvalerate (Position χ^2, $P < 0{\cdot}05$). Strains collected from the ICRISAT farm recorded only low-level resistance to cypermethrin (0·8- to 2-fold), while resistance to fenvalerate assayed on the 1995 strain was higher (2- to 19-fold). Ldp lines of six of the eight strains assayed with fenvalerate recorded systematic curvilinearity and significant heterogeneity (Heterogeneity χ^2, $P < 0{\cdot}01$), suggesting that these strains included a mixture of resistance phenotypes. Only three of 21 strains assayed with fenvalerate recorded significant heterogeneity (Heterogeneity χ^2, $P < 0{\cdot}05$). The highest cypermethrin resistance levels were recorded in 1996 (22- to 197-fold). In general in most years the highest pyrethroid resistance levels were recorded in strains from the coastal districts of Guntur and Prakasam and from Cuddapah.

In all field strains there was a marked synergism of cypermethrin toxicity with PBO resulting in synergist

TABLE 5
Toxicity of Topically Applied Endosulfan to 30–40-mg Larvae of Field Strains of *Spodoptera litura*

Strain[a]	*Collect date*	n	LD_{50}[b] (*95% C.I.*) (*μg per larva*)		LD_{90}	*Slope* (±*S.E.*)	*RF*[c]
Bangalore		240	1·2	(1·0–1·4)	3·1	3·1 (±0·4)	—
ICRISAT (US)	Jul. 93	384	3·9	(2·9–5·2)	37	1·3 (±0·1)	3
ICRISAT (US)	Feb. 95	384	1·5***	(1·1–2·0)	13	1·4 (±0·1)	1
Karlapalem (S)	Feb. 93	336	1·6*	(1·2–2·0)	9·7	1·6 (±0·2)	1
Gonapavarum (S)	Feb. 93	336	5·6**	(4·5–7·0)	26	1·9 (±0·2)	5
Bapatla (S)	Feb. 94	480	4·5**	(3·6–5·6)	28	1·6 (±0·1)	4
Bapatla (US)	Feb. 95	336	5·4	(4·1–7·4)	55	1·3 (±0·2)	5
Bapatla (S)	Feb. 95	288	6·1	(4·9–7·7)	31	1·8 (±0·2)	5
Proddatur (S)	Feb. 95	336	2·1*	(1·5–2·7)	16	1·4 (±0·2)	2
Tirupati (S)	Feb. 95	288	1·4	(1·1–1·7)	5·5	2·1 (±0·2)	1
Tirupati (US)	Feb. 95	336	2·3	(1·7–3·2)	31	1·1 (±0·2)	2
Bapatla (US)	Feb. 96	216	3·0*	(2·4–3·6)	8·9	2·7 (±0·3)	3
Bapatla (S)	Feb. 96	216	16***	(9·8–2·5)	211	1·1 (±0·1)	13
Proddatur (US)	Feb. 96	216	7·2	(5·6–9·5)	34	1·9 (±0·3)	6
Proddatur (S)	Feb. 96	180	3·1***	(2·1–4·0)	14	1·9 (±0·4)	3
Tirupati (US)	Feb. 96	144	1·8	(1·5–2·2)	3·8	3·9 (±0·6)	2
Tirupati (S)	Feb. 96	216	3·4***	(2·7–4·3)	13	2·2 (±0·3)	3

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
[c] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain.

TABLE 6
Toxicity of Topically Applied Monocrotophos to 30–40-mg Larvae of Field Strains of *Spodoptera litura*

Strain[a]	*Collect date*	n	LD_{50}[b] (*95% C.I.*) (*μg per larva*)		LD_{90}	*Slope* (±*S.E.*)	*RF*[c]
Bangalore		216	5·9	(4·4–7·8)	28	1·9 (±0·3)	—
ICRISAT (US)	Jul. 93	384	11	(8·4–15)	124	1·2 (±0·1)	2
ICRISAT (US)	Feb. 95	336	17	(12–23)	170	1·3 (±0·2)	3
Karlapalem (S)	Feb. 93	480	14	(10–21)	217	1·1 (±0·1)	2
Gonapavarum (S)	Feb. 93	336	15	(12–21)	142	1·3 (±0·2)	3
Bapatla (S)	Feb. 94	336	40	(29–54)	463	1·2 (±0·2)	7
Bapatla (US)	Feb. 95	288	22	(17–27)	107	1·8 (±0·2)	4
Bapatla (S)	Feb. 95	336	19	(14–25)	163	1·4 (±0·1)	3
Bapatla (US)	Feb. 96	180	243*	(157–721)	1565	1·6 (±0·4)	41
Bapatla (S)	Feb. 96	216	2134	—	—	0·5 (±0·3)	362
Proddatur (US)	Feb. 96	180	93	(69–136)	529	1·7 (±0·3)	16
Proddatur (S)	Feb. 96	180	455	(235–4488)	—	1·5 (±0·5)	77
Tirupati (US)	Feb. 96	180	51	(39–76)	268	1·8 (±0·4)	9
Tirupati (S)	Feb. 96	180	79**	(57–113)	488	1·6 (±0·3)	13

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.
[c] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain.

ratios of 2- to 121-fold at the LD_{50} level (Table 3). PBO pretreatment reduced LD_{50} levels close to those of the susceptible strain (RF = 0·2- to 2-fold).

3.3 Endosulfan resistance

The Bangalore strain recorded a steep ldp line slope and low LD_{50} (relative to field strains), indicating that the response of this strain was representative of the baseline susceptible response (Table 5). For all the field strains, only low-level incipient resistance was recorded (1- to 6-fold), with slope values ranging from 1·1 to 3·9. The only exception was one strain from Bapatla (13-fold resistance), collected from a field that had received 10 applications of insecticide, including at least two sprays of endosulfan. Heterogeneity was significant for nine out of the 16 strains tested (χ^2, $P < 0{\cdot}05$), indicating that many of these strains comprised a mixture of resistant and susceptible phenotypes. There was no association between LD_{50} responses and geographic location.

3.4 Organophosphate resistance

The Bangalore strain recorded the steepest slope (1·9) of all strains assayed with monocrotophos and the LD_{50} of 5·9 μg per larva was probably representative of the susceptible strain phenotype (Table 6). Resistance factors for field strains ranged from 2- to 362-fold. The highest resistance levels were reported in the 1996 strains collected from insecticide-treated groundnut crops in Guntur and Cuddapah districts. Pre-treatment of the Bangalore strain with DEF slightly inhibited the

TABLE 7
Effect of DEF in Suppressing Monocrotophos Resistance in 30–40-mg Larvae Derived from Field Strains of *Spodoptera litura*

		Monocrotophos alone		*Monocrotophos + DEF*			
Strain[a]	*Collect date*	LD_{50}[b] (*μg per larva*)	*Slope*	LD_{50}[b] (*μg per larva*)	*Slope*	*RF*[c]	*SR*[d]
Bangalore		5·9	1·9	8·1	1·4	—	0·7
ICRISAT (US)	Feb. 95	17	1·3	6·6	1·8	0·8	3
Bapatla (US)	Feb. 95	22	1·8	11**	1·1	1	2
Bapatla (S)	Feb. 95	19	1·4	12	1·6	1	2

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: ** $P < 0{\cdot}01$.
[c] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain (both pretreated with DEF).
[d] SR (synergism ratio) = LD_{50} without DEF/LD_{50} with DEF pretreatment.

TABLE 8
Toxicity of Topically Applied Quinalphos to 30–40-mg larvae of Field strains of *Spodoptera litura*

Strain[a]	*Collect date*	n	LD_{50}[b] (*95% C.I.*) (*μg per larva*)		LD_{90}	*Slope* (±*S.E.*)	*RF*[c]
Bangalore		288	0·12	(0·10–0·15)	0·52	2·0 (±0·3)	—
ICRISAT (US)	Jul. 93	288	0·33	(0·27–0·41)	1·5	2·0 (±0·2)	3
ICRISAT (US)	Feb. 95	240	0·11	(0·10–0·13)	0·24	4·0 (±0·5)	1
Karlapalem (S)	Feb. 93	384	0·48*	(0·40–0·58)	1·7	2·3 (±0·2)	4
Gonapavarum (S)	Feb. 93	336	0·22*	(0·17–0·28)	1·2	1·8 (±0·2)	2
Bapatla (S)	Feb. 94	480	0·60***	(0·46–0·79)	8·1	1·1 (±0·1)	5
Bapatla (S)	Feb. 95	240	0·71	(0·58–0·89)	2·6	2·3 (±0·3)	6
Proddatur (US)	Feb. 95	288	0·20	(0·16–0·25)	0·89	2·0 (±0·2)	2
Tirupati (US)	Feb. 95	288	0·22	(0·19–0·27)	0·67	2·7 (±0·3)	2
Tirupati (S)	Feb. 95	336	0·13	(0·10–0·17)	0·82	1·6 (±0·2)	1
Bapatla (US)	Feb. 96	216	0·78	(0·60–1·0)	4·0	1·8 (±0·3)	7
Bapatla (S)	Feb. 96	216	0·83	(0·68–1·0)	2·6	2·6 (±0·3)	7
Proddatur (US)	Feb. 96	216	3·5	(2·7–4·7)	18	1·8 (±0·3)	29
Proddatur (S)	Feb. 96	216	0·97**	(0·72–1·4)	6·7	1·5 (±0·3)	8
Tirupati (US)	Feb. 96	216	0·22	(0·18–0·28)	0·75	2·4 (±0·3)	2
Tirupati (S)	Feb. 96	216	0·15***	(0·12–0·19)	0·47	2·6 (±0·3)	1

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
[c] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain.

toxicity of monocrotophos but this was not statistically significant (Position χ^2, $P > 0{\cdot}05$) (Table 7). For the three field strains, DEF significantly synergised monocrotophos toxicity (Position χ^2, $P < 0{\cdot}05$), with synergist ratios ranging from 2 to 3.

It was not clear whether or not the Bangalore strain was truly susceptible to phosphorothionate organophosphate insecticides, as the ldp line slope for quinalphos was only moderately steep (2·0) compared to some of the field strains (range: 1·1 to 4·0) (Table 8). However, the LD_{50} of 0·12 μg per larva seemed a reasonable estimate of the baseline response for quinalphos, as only one of the field strains recorded a lower LD_{50}. In general, only low-level incipent resistance was indicated (1- to 8-fold). A single strain from Prodattur recorded 29-fold resistance to quinalphos.

3.5 Methomyl resistance

The Bangalore strain recorded the steepest slope (3·0) of all the strains assayed and it is probably reasonable to assume that it is representative of the baseline susceptible response (Table 9). Resistance in field strains ranged from 0·7- to 19-fold, indicating only low-level

TABLE 9
Toxicity of Topically Applied Methomyl to 30–40-mg Larvae of Field Strains of *Spodoptera litura*

Strain[a]	*Collect date*	n	LD_{50}[b] (*95% C.I.*) (*μg per larva*)		LD_{90}	*Slope* (±*S.E.*)	*RF*[c]
Bangalore		192	0·46	(0·37–0·55)	1·2	3·0 (±0·4)	—
ICRISAT (US)	Feb. 95	384	0·32	(0·25–0·42)	2·6	1·4 (±0·1)	0·7
Bapatla (US)	Feb. 95	336	0·74	(0·56–0·97)	6·1	1·4 (±0·2)	2
Proddatur (S)	Feb. 95	288	0·87	(0·72–1·1)	3·2	2·3 (±0·3)	2
Tirupati (US)	Feb. 95	336	1·4**	(1·0–1·9)	19	1·1 (±0·1)	3
Tirupati (S)	Feb. 95	336	2·0***	(1·5–2·7)	17	1·4 (±0·2)	4
Bapatla (US)	Feb. 96	324	2·2**	(1·5–3·3)	51	0·9 (±0·1)	5
Bapatla (S)	Feb. 96	288	1·7***	(1·0–2·6)	49	0·9 (±0·1)	4
Proddatur (US)	Feb. 96	288	6·4*	(4·6–9·5)	72	1·2 (±0·2)	14
Proddatur (S)	Feb. 96	216	8·7*	(7·1–11)	25	2·8 (±0·4)	19
Tirupati (US)	Feb. 96	288	0·86	(0·60–1·2)	13	1·1 (±0·2)	2
Tirupati (S)	Feb. 96	252	1·4	(1·0–1·9)	13	1·3 (±0·2)	3

[a] (S) = collection from sprayed fields; (US) from unsprayed fields.
[b] Heterogeneity χ^2 significant at: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
[c] RF (resistance factor) = LD_{50} Field Strain/LD_{50} Bangalore strain.

incipient resistance to methomyl. In general, the results paralleled those obtained with quinalphos.

4 DISCUSSION

Results of this study clearly demonstrate that *S. litura* populations in many regions of Andhra Pradesh have developed some level of resistance to various classes of insecticide, including pyrethroids, endosulfan, some organophosphates and carbamates. This is the first time that resistance to synthetic pyrethroid insecticides has been confirmed in *S. litura* in India; however, Ramakrishnan *et al.*,[5] had earlier reported a 15-fold increase in tolerance to natural pyrethrum between strains collected in 1961 and 1983.

Resistance to pyrethroids was particularly high in 1996 in Guntur district. The exact reasons for this cannot be traced, although it is known that there was a heavy infestation of *S. litura* on early-sown crops in November and December throughout the study area. An IPM implementation program involving minimum insecticide application has started in the district, but it is too early to expect the impact required of an area-wide insecticide resistance management program. Tobacco is grown in the vicinity of the major groundnut growing area and the nurseries receive much insecticide to protect the plants from *S. litura* attack from December onwards. The authors suspect that these nurseries are the primary source of the infestations affecting groundnut in January–March each year.

The reason for the higher resistance factors reported for fenvalerate compared to cypermethrin are not clear, as both insecticides are used by farmers for *S. litura* control. Brewer and Trumble[10] reported a similar phenomenon for *Spodoptera exigua* (Hübner) where fenvalerate resistance ratios were significantly higher than those for permethrin. They concluded that the difference was attributable to the fact that growers commonly used fenvalerate but not permethrin. This explanation does not hold for India where both cypermethrin and fenvalerate are commonly used by farmers for *S. litura* control. Overall the data indicate a clear increase in pyrethroid resistance levels between 1991 and 1996.

In nearly all the strains tested, pre-treatment with the synergist PBO completely restored susceptibility to cypermethrin. It is likely, therefore, that enhanced detoxification by microsomal P450-dependent monnoxygenases was the major mechanism of pyrethroid resistance in *S. litura* populations in Andhra Pradesh.

Endosulfan resistance levels were low, although the marked resistance heterogeneity in many of the strains assayed suggests that resistant phenotypes were present to varying degrees in most populations. Our results differ from those of Ramakrishnan *et al.*,[5] who reported a 56-fold increase in endosulfan resistance between the early 1970s and 1983 and concluded that endosufan resistance levels in Andhra Pradesh were high.

Only low-level resistance to quinalphos was found, as was the case for monocrotophos up until 1996, when resistance increased markedly. As DEF is an inhibitor of esterases and the glutathione *S*-transferase system, the 2- to 3-fold synergism with monocrotophos indicates that these mechanisms are at least to some extent likely to be contributing to organophosphate resistance. The fact that full suppresion of resistance was never achieved with DEF suggests that at least one other mechanism was conferring organophosphate resistance. Classically, resistance to the phosphate type organophosphates (e.g. monocrotophos), is attributed to insensitive acetylcholinesterase (AChE) mechanisms.[11] Saratchandrudu *et al.*[12] found regional differences in AChE titres in *S. litura* strains in Andhra Pradesh. They correlated these differences with insecticide use and concluded that AChE was an important organophosphate resistance mechanism in regions where there was heavy reliance on insecticides for *S. litura* control. In *Spodoptera littoralis* Boisd. in Egypt, esterases have been found to be the major organophosphate resistance mechanism.[13,14]

Methomyl resistance levels were generally low (0·7- to 4-fold), except for the two strains collected from Proddatur in 1996. It is possible, however, that even low-level methomyl resistance could result in field control difficulties, as has been reported for *Helicoverpa armigera* (Hübner).[15] Earlier resistance reports have shown that *S. litura* was immune to field application rates of the carbamate insecticide carbaryl by 1971 in a north India strain[4] and by 1983 in Andhra Pradesh.[5] As the mechanism of methomyl resistance is unknown, the possibility of cross-resistance between carbaryl and methomyl cannot be confirmed.

It can be concluded from this study that *S. litura* in Andhra Pradesh has developed significant levels of resistance to cypermerthrin, fenvalerate and monocrotophos, but resistance to endosulfan, quinalphos and methomyl was in most instances moderate or low. It appears, however, that resistance may be increasing with time as indicated by the generally high resistance levels to all chemical classes reported in strains tested in 1996. The generally high levels of heterogeneity among field populations indicate that the continuing application of insecticides should be monitored and as far as possible moderated, if the 'sudden' appearance of populations highly resistant to a range of insecticide classes is to be avoided.

ACKNOWLEDGEMENTS

We are indebted to Dr G. Shankar of Rallis India Ltd, for providing the Bangalore strain. Thanks are due to Mr Madhusudhan Reddy for rearing the *S. litura* strains and assisting with the bioassays. K. Babu Rao, A. Gafoor and I. Nemiligundam kindly assisted with field collection of *S. litura* strains at various times.

Financial support to NJA was provided by the Natural Resources Institute, UK, through an Adaptive Research Initiative of the UK Governments's Overseas Development Administration.

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